THE OSMOTIC RELATIONS BETWEEN FISHES AND THEIR SURROUNDING MEDIUM (PRELIMINARY NOTE).[1]

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The effects upon fishes of changes in the density and salinity of the surrounding medium involve numerous problems of great physiological importance. Why is an extreme change of density so fatal in some cases and so harmless in others? And is it the change of *density* which is responsible for the harmful effects after all? May not salt water be *toxic* in a narrower sense to fresh-water fishes and *vice versa?* In any case, what is the immediate cause of death? Are the limiting membranes of a fish permeable to both water and salts, or are they only semi-permeable? Or are they perhaps impermeable to both? And are all of the limiting membranes alike in this regard? Likewise is their condition the same for all species and under all circumstances? These are closely related questions. They have received many and quite contradictory answers. It is hoped that the experiments here discussed have contributed something toward their solution.

The first of these experiments were chiefly concerned in determining whether a given change in water density was harmful to a given species of fish, records being kept of the rate of death. In a second series, weight determinations were made with a view to ascertaining whether such changes in the density of the surrounding medium were accompanied by appreciable osmotic effects upon the fishes. Third, it was sought to discover whether the membranes were permeable to water only or to salts as well. The passage of salts from the fishes into the surrounding water was tested chemically, and likewise the salt content of the tissues of fishes of several species under different conditions was determined. Finally a series of experiments was performed with a view to discovering whether such osmotic changes were confined

[1] A more complete account of these experiments is in course of publication by the Bureau of Fisheries and will, before long, appear in the Bulletin of that bureau.

to the gills or whether the general integument of the body were likewise concerned.

Altogether the results of about 150 experiments have been taken into consideration in arriving at the conclusions here presented. These experiments were carried on during the summers of 1904 and 1905 at the biological laboratory of the Bureau of Fisheries at Woods Hole, Mass., and during the spring of 1905 at the New York Aquarium. Acknowledgments are due to the officials of the Bureau of Fisheries for facilitating the progress of this work; and to the director of the New York Aquarium, Mr. Chas. H. Townsend, who placed at my disposal a room equipped for research, and provided me with abundant material throughout. My thanks are likewise due to Prof. W. C. Sabine, of the department of physics of Harvard University, for valuable criticism, and to Mr. D. W. Davis, assistant at the Fisheries Laboratory, for help during the earlier portion of the work.

Full details of these experiments, including the methods employed, and the precautions taken, must be deferred to the more extended paper which will soon appear. In the meantime, the principal results may be summarized as follows:

1. *Certain brackish and salt-water fishes were unable to survive even a gradual transfer to pure fresh water, though enduring an abrupt transfer to water of a very low degree of salinity. Thus fresh water, as such, proved fatal to these fishes, the degree of abruptness of the change being of secondary importance.*

These conclusions are drawn from experiments upon the three local species of killifishes (*Fundulus heteroclitus*, *majalis* and *diaphanus*), together with the allied species, *Cyprinodon variegatus*; likewise the white perch (*Morone americana*), cunner (*Tautogolabrus adspersus*), tautog (*Tautoga onitis*), sculpin (*Myoxocephalus octodecimspinosus*), and winter flounder (*Pseudopleuronectes americanus*). The death of a varying (often a large) proportion of specimens of *F. diaphanus*, when transferred from mildly brackish (sp. gr. 1.002–1.006) to pure fresh water was certainly unexpected, since this species in nature is not confined to brackish waters, but is indigenous to lakes and streams far from the coast. *F. heteroclitus*, likewise, is known to occur at times in fresh water; but the writer has found (*contra* Garrey[1]) that in nearly every

[1] BIOLOGICAL BULLETIN, Mar., 1905.

experiment the entire lot died throughout a period of from less than a day to several weeks.

In addition to a simple reversal of the normal medium (salt-water fishes in fresh water and *vice versa*) experiments were conducted upon *acclimatization*, which was found to retard, but not to prevent, the fatal effects of fresh water; likewise with *water of a very low degree of salinity*, which gave some of the most striking results to be recorded; with *fresh and salt water in alternation*, in which case the fatal effects of the former were diminished or annulled; and with *distilled water*, which soon proved fatal to *F. heteroclitus* (the only species used). *Surface abrasions* (extensive removal of scales) hastened the death of *F. heteroclitus* in fresh water, but only exceptionally led to the death of fishes returned to full-strength sea water (again, *contra* Garrey)[1], and wrought no harm to fishes placed in very dilute brackish water (three to four per cent. sea water), despite the fact that the latter was without doubt strongly hypotonic to the fish.

2. *Considerable changes of weight were found to result, in many cases, from changes in the salinity (hence the osmotic pressure) of the surrounding medium.*

The weighing operations were conducted with the following species: *Fundulus heteroclitus*, *F. majalis*, *F. diaphanus*, *Myoxocephalus octodecimspinosus*, *Microgadus tomcod*, *Pseudopleuronectes americanus*, *Stenotomus chrysops*, *Ameiurus nebulosus*, *Leuciscus erythrophthalmus*, *Morone americana* and *Oncorhynchus tschawytscha*. During these experiments, the fishes were kept unfed, and it is needless to add that abundant control experiments were performed in order to determine the normal rate of loss through waste. The changes of weight following changes in the density of the surrounding medium were frequently surprisingly great, at times as much as five per cent. or more in a single day. In many cases, moreover, they were not accompanied by any apparent harmful effect upon the fishes. With a very few exceptions, the changes were such as to indicate that they were the result of osmotic action. Thus, as a rule, the fishes *gained* in weight only in solutions known to be decidedly hypotonic to their body fluids, while with few exceptions a significant *decrease* only occurred in

[1] *Op. cit.*

those cases in which they were transferred from a hypotonic or isotonic medium to one which was strongly hypertonic. Negative results were indeed encountered at times, but very few which could be regarded as contradictory.

Extensive *surface abrasions* did not facilitate the influx or efflux of water. The changes of weight in *dead* fishes were such as to show that factors other than osmosis were concerned. Dead fishes of most of the species used were found to gain in weight in water of any degree of salinity up to the strength of normal sea water.

3. *Considerable changes in the salt (chlorine) content of the body were likewise found to result, in many cases, from changes in the salinity of the water.*

The problem here involved was attacked from both sides. In the first place, the passage of salts (strictly speaking, of *chlorides*)[1] into fresh water from fishes taken from salt or brackish water was tested chemically. In the second place, the salt content of the tissues of various fishes which had lived in water of various degrees of salinity was likewise determined.[2] It was found that the results from these two methods presented some striking points of agreement, though the latter proved, on the whole, to be much more satisfactory. These changes in the chlorine content of the body were frequently astonishing in their magnitude. *F. heteroclitus* by both methods was found to part with about twenty-five per cent. of its chlorine in the course of a single day. The loss of chlorides from the body was, however, found to occur at a steadily diminishing rate.

The following table indicates the percentages of chlorine found in specimens of *F. diaphanus* from brackish water (their habitat locally) and in those kept for varying periods in fresh and in salt water. The series is certainly suggestive.

Fresh water	11 days (8 fishes employed)	0.085
	3 days (4 fishes employed)	0.108
	1 day (4 fishes employed)	0.112

[1] Mohr's silver nitrate titration method was here employed. The gain or loss in the proportion of chlorine was held to be indicative of the behavior of the various saline ingredients of sea water.

[2] It is needless to state that all fishes used in these two series of tests were previously thoroughly rinsed in fresh water.

Brackish water (sp. gr., 1.002) (5 lots of 4 fishes each), 0.134 [or 0.142][1]

Salt water (1.023)	5 days (2 fishes employed)	0.143
	10 days (3 fishes employed)	0.151

It will be seen that the last of these figures is about 78 per cent. greater than the first! And yet the fishes were all alive and apparently well at the time they were killed for analysis. It will be likewise seen that whichever figure be regarded as the more correct one for the brackish-water fishes, the latter agree much more closely with the salt-water than with the fresh-water individuals (the comparison being of course between the extreme members of the series). It must be added in strict fairness, however, that in two different tests fishes kept for only *one* day in sea-water gave a much higher percentage of chlorine than those kept for five or ten days. For this apparent anomaly I believe that a satisfactory explanation can be given, but this has been deferred to my longer work.

A series of figures somewhat similar to the above was obtained from experiments with the white perch.

Experiments with both *F. heteroclitus* and *F. majalis* agreed in showing a great difference between the effects upon the chlorine content of the body of pure fresh water and water having a certain small percentage of salt. This difference is extremely significant in view of the difference, already mentioned, in their effects upon the life of the fishes. Moreover, it was further found that the average percentage of chlorine contained in the salt-water fishes analyzed was of the same order of magnitude as that of water containing just enough salt to maintain the fishes in health. That such water could not have been even approximately *isotonic* with the body fluids of these fishes seems evident from the cryoscopic determinations of other investigators.

4. *Careful control experiments excluded the possibility that the water or salts entered or passed from the body through the alimentary canal, leaving as the only probable alternative an osmotic exchange through one or more of the external membranes.*

The alimentary canal, and indeed the whole abdominal viscera, together with the washings from the body cavity, were found in

[1] This second figure is the mean which results when one very questionable determination is included. It is inserted for the sake of strict fairness. The other averages are in each case derived from *all* of the fishes tested.

some cases to yield less chlorine than passed from the body of a living fish in the course of a few hours. Likewise those fishes whose bodies were analyzed gave approximately the same percentages of chlorine whether or not the alimentary canal was included in the analysis. It must be remembered also that the fishes employed in these experiments had, in all cases, been kept unfed for some days previously.

5. *In certain fishes, at least, it was found that the membranes chiefly concerned in such exchanges were those of the gills.*

In the case of certain specimens, salt water was passed through the gills by means of a rubber tube placed in the mouth, the body being bathed with fresh water; while in others the arrangement was reversed, the gills receiving fresh water and the general integument salt. In six experiments with the carp it was found that a considerable loss of weight occurred in all of those cases in which the former conditions obtained, while the weight remained practically stationary in those cases in which the conditions were reversed.

A complete historical review of previous researches in this field of physiology would be beyond the scope of the present paper. The investigations of Fredericq,[1] Bottazzi,[2] Rodier,[3] Garrey[4] and Greene[5] agree in showing that the blood of marine teleosts is far from being isotonic with the surrounding sea-water, but that it has an osmotic pressure which is roughly about one half that of the latter. But it has likewise been shown (Fredericq, Greene, *op. cit.*) that the osmotic pressure of the blood of salt-water teleosts is considerably higher than that of fresh-water ones, though this fact has been almost lost sight of in the zeal to prove that the internal medium is not isotonic with the external, and that its osmotic pressure is *relatively* constant. Indeed it does not seem to have been generally appreciated that there is a certain correlation between the inner and outer fluids, both as regards osmotic pressure and salt content; and certain authors have been free to state that the membranes of teleost fishes form an effective barrier against osmotic changes. Fredericq makes this assertion

[1] *Archives de Biologie*, 1904.

[2] *Archives italiennes de Biologie*, 1897.

[3] Cited by Fredericq, 1904.

[4] BIOLOGICAL BULLETIN, 1905.

[5] Bulletin U. S. Bureau of Fisheries, 1905.

broadly, while Garrey says of *Fundulus heteroclitus*: "The integument and gills are therefore impermeable." Garrey is cautious enough, however, not to postulate an *absolute* impermeability, either for *Fundulus* or for teleosts in general. Greene, though he finds that the osmotic pressure of the blood of the Pacific salmon undergoes a decrease of about 17½ per cent. when the fish ascends a river to spawn, is nevertheless doubtful whether osmotic exchanges with the surrounding water are responsible for this decrease.

In view of my own experiments, however, we are certainly not justified in concluding from the absence of osmotic equilibrium between the fish and its environment that no osmotic interchanges normally occur. On the contrary, abundant experiments seem to prove that both water and salts may, under certain conditions, be transmitted in either direction without any harm resulting to the fish. These conditions seem impossible to state in advance for a given case. In general we may say that:

1. *Measurable changes in weight result only from considerable changes in the surrounding water, but*—

2. *Not all such changes of density suffice to produce changes of weight, even when the fish is transferred to a medium which is known to be strongly hypertonic or hypotonic to its own body fluids.*

3. *Changes in the salinity of the water may or may not result in changes in the salt content of the body.*

4. *Changes in the bodily salt content may or may not be accompanied by changes in weight.*

5. *Neither the changes in weight nor in salt content are at all proportional to the changes in the density of the external medium.*

It would appear that there is normally a tendency on the part of the fish to resist osmotic changes and to maintain the fluids of the body at a definite degree of concentration. Under various conditions, however, this resistance is overcome and a certain degree of permeability is established. This is generally a differential permeability, resulting in osmosis and consequent changes of weight. In such cases, however, the membranes are not strictly semipermeable, but transmit salts in some measure. Indeed it would seem that at times the permeability is indiscriminate, in which case the salts may diffuse freely, but no changes in weight occur. These various

changes continue until a new level of stability is established, after which the normal resisting power of the fish reasserts itself and no further alteration occurs as long as the medium is constant. Complete osmotic equilibrium between the fish and the water is probably never attained except in waters having roughly a medium degree of salinity. The osmotic pressure of the "internal medium" fluctuates within a much narrower range than that of the "external medium."

The foregoing conclusions are intended to apply only to normal fishes. It seems certain that the enfeeblement of the fish may result in an increased permeability of the membranes, which, in turn, would doubtless result in a further enfeeblement of the fish. The death of those fishes which cannot withstand transfer to a medium very different from that to which they are accustomed is thus probably in part a cause and in part an effect of these changes. Death is accompanied (perhaps in some cases caused) by a giving way in the power to resist an abnormal degree of osmotic exchange. The body becomes water-soaked (if in fresh water) or dehydrated (if in salt). The difference between the more hardy and the more delicate species in this regard seems to lie partly in the resisting power of the limiting membranes (chiefly those of the gills); partly, also, in internal differences, such as composition of blood, etc., which determine whether or not a given influx or efflux of water or salts shall prove fatal.

The actual cause of death following a change in the salinity of the water seems to differ in different cases. With those fishes which succumb rapidly with but a slight change of weight (*e. g.*, scup), it is unlikely that any appreciable alteration occurs in the tissues at large. Such changes are probably confined to the blood, perhaps, as Bert[1] held, to that in the gill capillaries, in which case death may result from asphyxiation (Bert,[2] Mosso[3]). In those cases, on the contrary, where the fatal effects are not manifested for some days, it seems likely that the manner of death is different. In the case of *F. heteroclitus*, it was found in most instances that the endosmotic flow of water had ceased, and that a secondary *decrease* in weight had ensued, within one

[1] *Comptes Rendus de l'Académie des Sciences*, 1871, 1883. [2] *Op. cit.*
[3] *Biologisches Centralblatt*, 1890.

or two days after transfer to fresh water. On the other hand, it will be remembered that fishes of this species commonly did not die for a considerable number of days, while many survived for a week and some even for several weeks. Again, it will be recalled that the fatal effects of fresh water upon this and some other species were nullified by the admixture of a very small percentage of salt water. Analyses showed that in this latter case there was little or no decrease in the salt content of the body. A rough approximation was pointed out between the percentage of salts in this faintly saline water and that in the fishes themselves. *All of these facts point to the conclusion that one factor in the death of salt-water fishes in fresh water is the extraction from their tissues of an amount of salts sufficient to reduce the percentage below a certain necessary minimum.*

If the question be asked: Why are not fresh-water fishes thus affected in their own medium? it is replied that their membranes have been adapted to resisting such an extraction of salts. It is perhaps also true that the irreducible minimum of salts in these species is lower than in the case of salt-water ones. In any case, the percentage actually present is, on the average, less (Atwater,[1] Katz,[2] Quinton[3] and several others).

Whether or not salt water ever has a *toxic* effect, in the narrower sense, upon fresh-water fishes cannot be stated definitely. Bert denied that such was the case, but he is not entirely consistent in this position. In view of the fatal effects upon salt-water fishes of some of the individual components of sea salt, when taken separately (Loeb,[4] Siedlecki[5]), it seems quite possible that sea-water may act as a poison to fresh-water organisms, independently of any osmotic effects. Indeed both of the last-named writers have shown that it is the chemical nature of the solutions used rather than their osmotic pressures which determines, in many cases, whether or not they shall prove fatal.

College of the City of New York,
March 27, 1906.

[1] Report U. S. Com. Fish and Fisheries for 1888 (1891).

[2] *Archiv für die gesammte Physiologie*, 1896.

[3] "L'eau de mer, milieu organique," Paris, 1904.

[4] *American Journal of Physiology*, 1900.

[5] *Comptes Rendus de l'Académie des Sciences*, 1903.